constants (τ) of calcium-transient decay among cell neighborhoods at baseline and during stimulation of either beta-adrenergic receptors or cholinergic receptors.
Beta-adrenergic receptor stimulation (isoproterenol) not only decreased cycle length (CL) and average τ vs. baseline, but also decreased the standard deviation (SD) of all τ's across all neighborhoods, suggesting a shift into a less heterogeneous, i.e. more synchronized calcium pumping throughout SANC. Conversely, cholinergic receptor stimulation (carbachol) not only increased CL and average τ vs. baseline, but also increased the local SD(τ), suggesting a shift into a more heterogeneous, i.e. less synchronized calcium pumping within SANC. Furthermore, on a beat-to-beat basis, the relationship between either τ or SD(τ) and CL was linear under both baseline conditions and autonomic stimulation.
Conclusions: The degree of heterogeneity of local calcium pumping is a new universal factor that affects the CL and insures effective rate and rhythm regulation of the coupled-clock pacemaker system via autonomic modulation. More synchronized and faster calcium pumping is presumably achieved via phospholamban phosphorylation and allows cell neighborhoods to reach the calcium release threshold quicker and more synchronously, thereby synchronizing LCRs and amplifying their ensemble diastolic signal, accelerating pacemaker rate.

2884-Pos Board B314
Excitation-Metabolism Coupling in Mouse Heart
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A beating heart must balance energy consumption with production. This balance requires cellular ATP production to respond dynamically to changes in cardiac output and energy demand. While it is well understood that mitochondrial oxidative phosphorylation serves as the primary pathway for ATP production in heart, the mechanisms that govern the regulation of mitochondrial metabolism remain unclear. Calcium (Ca^{2+}), specifically Ca^{2+} in the mitochondrial matrix ($[Ca^{2+}]_m$), has been implicated as a likely signaling pathway for this regulation. $[Ca^{2+}]_m$ has been suggested to regulate nearly every stage of mitochondrial metabolism including the activity of the tricarboxylic acid cycle (TCA) via Ca^{2+}-sensitive dehydrogenases (DHOs), critical proteins in the electron transport chain (ETC), and even the F_1/F_0 ATP synthase itself. Here we expand our local control mathematical model of excitation-contraction coupling in mouse heart to investigate excitation-metabolism coupling. The model features mechanistic initiation and termination of Ca^{2+} sparks and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) transients in a system that maintains sarcoplasmic reticulum (SR) Ca^{2+} pump/leak balance. Mitochondrial Ca^{2+} uptake and export is simulated using experimentally constrained formulations of the mitochondrial Ca^{2+} uniporter (MCU) and the mitochondrial sodium/Ca^{2+} exchanger (NCLX). The model employs key mechanisms of ATP consumption (i.e. SR Ca^{2+} ATPase and myosin ATPase) and buffering (phosphocreatine) as well as a Ca^{2+} dependent model for mitochondrial ATP generation. We investigated mitochondrial Ca^{2+} dynamics at physiological pacing frequencies for mouse heart and show that changes in ATP consumption can be translated to increased energy production through $[Ca^{2+}]_m$ signals. Our model provides new insights into mitochondrial Ca^{2+} dynamics and how these $[Ca^{2+}]_m$ signals may function to preserve energy homeostasis in the face of increased demand.

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Similarities and Differences in Gating of the Two-Pore Channels TPC1 and TPC2
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Endolysosomal Ca^{2+} homeostasis is implicated in several diseases and controls many endolysosomal functions. A key to understanding endolysosomal Ca^{2+} signaling to understand the function of the newly discovered endolysosomal $PI(3,5)P_2$-regulated two-pore channels (TPCs) and their potential activation by NAADP. Our recent work concluded that the lysosomal TPC2 function as a NAADP and $PI(3,5)P_2$-activated channel regulated by cytoplasmic Mg^{2+}. The properties and gating of the mostly endosomal TPC1 are not known. Recording whole-organelle currents of enlarged endosomes expressing GFP-TPC1, we discovered that TPC1 is potently activated by $PI(3,5)P_2$ and NAADP. Moreover, $PI(3,5)P_2$ facilitates TPC1 activation by NAADP. Unlike, TPC2, the activity of TPC1 showed poor inhibition by Mg^{2+}. Most notably, the concentration dependence of activation of TPC1 and TPC2 by NAADP are remarkably different. While activation of TPC2 by NAADP followed normal saturation dependence with no sign of inhibition by high NAADP concentration, activation of TPC1 by NAADP followed a bell shaped dependency, resembling the NAADP-mediated Ca^{2+} release in intact cells. These findings provide the first direct evidence for gating of TPC1 by NAADP and indicate that endosomal, rather than lysosomal, Ca^{2+} release is the key trigger Ca^{2+} pool that sensitizes the IP_3-mediated Ca^{2+} release from the ER. The interaction between TPC1-expressing endosomes and the ER are being examined.

2886-Pos Board B316
Mitochondrial Calcium and Bioenergetics Controlled by Tight Coordination of MCU and NCLX
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Mitochondrial Ca^{2+} is regulated by Ca^{2+} transport proteins in the mitochondrial inner membrane. Among these are the mitochondrial calcium uniporter (MCU), a Ca^{2+} channel responsible for most Ca^{2+} uptake in mitochondria, and NCLX, a Na^+/Ca^{2+} exchanger, mediating Ca^{2+} release to the cytosol. After more than five decades of intensive study, the MCU gene was finally cloned in 2010. Strikingly, MCU-KO mouse, whose mitochondria lose >90% Ca^{2+} uptake capacity, was born normally with very mild phenotype and only slight reduction of mitochondrial Ca^{2+}. How does the animal maintain normal physiology without MCU, which is highly conserved and tightly regulated in most eukaryotes?
To address this issue, we use an MCU-KO HEK293T line as our model system. Consistent with animal experiments, these cells have normal mitochondrial morphology, matrix $[Ca^{2+}]$, and ATP production via oxidative phosphorylation. Interestingly, we observed a slow Ca^{2+} uptake in MCU-KO mitochondria, operating at 5-10% of the MCU capacity, resistant to a potent MCU inhibitor Ru360. Furthermore, these mitochondria lose Na^+-dependent Ca^{2+} release, due to decreased expression of the NCLX protein. We therefore propose that mitochondria compensate the loss of MCU by invoking a backup Ca^{2+} uptake mechanism, and by suppressing Ca^{2+} release. Supporting this hypothesis, we show that expressing NCLX in MCU-free mitochondria drastically reduces matrix $[Ca^{2+}]$, and abolishes >80% ATP output. Moreover, overexpression of WT-NCLX, but not a loss-of-function mutant S468A-NCLX, induces rapid death of MCU-KO cells.
We're now investigating how mitochondria invoke these compensatory mechanisms. Na^+/Ca^{2+} exchange can be restored by introducing human MCU gene into MCU-KO cells, but not by expressing transport-capable MCU homologues from A. thaliana or D. Discoideum, implicating that mitochondria might balance Ca^{2+} uptake and release through an MCU-NCLX interaction that stabilizes inner-membrane expression of NCLX.

Nucleo-Cytoplasmic Transport

2887-Pos Board B317
Observing Signal Transduction Directly at the Single-Molecule Level in Live Eukaryotic Cells
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All cells dynamically sense their environment through signal transduction mechanisms, allowing them to respond to environmental changes at the genetic level. The glucose sensory mechanism in Sacchoromyces cerevisiae is a model system of signal transduction. It uses the multi-copy inhibitor of the GAL gene expression Mig1 protein to repress unwanted genes in the presence of elevated level of extracellular glucose. In wild-type cell strains, and in genetically and biochemically impaired signal transduction phenotypes, we have fluorescently labelled the Mig1 protein with the green fluorescent protein GFP via chromosomal integration at native levels of expression, in addition to the RNAP nuclear reporter protein Nrd1 with the red fluorescent protein mCherry. [1] Using millisecond dual-color fluorescence excitation with Slimfield microscopy, [2] we track the fate of single molecules of Mig1 in live yeast cells at millisecond timescales. We present data showing a consistent difference between confined, high stoichiometry assemblages of bound Mig1 in the nucleus and freely diffusing single Mig1 molecules across the entire cell as well as more complex behavior in Mig1 traversing the nuclear membrane. The mobility and stoichiometry distribution of Mig1 assemblages we observe is functionally dependent on the signal transduction pathway and the concentration of extracellular glucose, allowing us to directly observe gene regulatory events.
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